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10/758,401	01/15/2004	G. Mike Makrigiorgos	700157-53471	6102

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EXAMINER	
BAUGHMAN, MOLLY E	
ART UNIT	PAPER NUMBER
1637	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/29/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/758,401	MAKRIGIORGOS, G. MIKE	
	Examiner	Art Unit	
	Molly E. Baughman	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 December 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *2 and 6-17* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *2 and 6-17* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *05 January 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/4/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election without traverse of Group II, claims 2, and 6-17, drawn to a method of amplifying a nucleic acid sequence of interest, and amendments to the elected claims in the reply filed on 12/5/2006 are acknowledged.

2. Cancellation of claims 1, and 18-34 is acknowledged.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 2, and 6-17 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a. Claim 2 is confusing because it cannot be determined what is encompassed by "a first primer that binds to at least a portion of the upper single stranded non-complementary region, and a second primer that binds to at least a portion of the lower single-stranded non-complementary region." The method of the claim comprising option (2) of the claim does not encompass such upper and lower single-stranded non-complementary regions, and as such, renders the claim confusing.

b. Claims 2, and 6-17 are confusing because it cannot be determined what is encompassed by "a hairpin DNA structure." Clarification is required.

c. Claims 6-17 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential elements, such omission amounting to a gap between the elements. See MPEP § 2172.01. The omitted elements are: what is encompassed by "(a) converting the sequence of interest into a hairpin DNA structure." While claims 7-13, and 15-17 do not particularly use the phrase, they depend from claims which use the phrase.

d. Claim 6 and 14 recite the limitation "the upper single stranded region" and "the lower single stranded region." There is insufficient antecedent basis for these limitations in the claim.

e. Claim 8 recites the limitation "the double stranded region" in claim 6. There is insufficient antecedent basis for this limitation in the claim.

f. Claim 11 is confusing because it cannot be determined what is encompassed by "a mismatched structure," and the specification does not provide further clarification.

g. Claim 11 is confusing because it cannot be determined what is encompassed by "a matched structure," and the specification does not provide further clarification.

h. Claim 13 is confusing because it cannot be determined how the claim further limits claim 12.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claim 2 is rejected under 35 U.S.C. 102(b) as being anticipated by Weissman et al. (U.S. 6,235,502).

Weissmen et al. describe a method of ligating hairpin forming adaptors to the ends of DNA fragments for rolling circle amplification, where the hairpins have 3' and 5' ends that are complementary to each other and form stem and loop structures, thereby forming a hairpin structure with the target (abstract, and column 4, lines 57-62). Adaptors can also be linear or Y shaped (Figure 1 A, column 3, lines 22-26, column 6, lines 42-63). Primers to the non-complementary sequences of the Y shaped adaptor, or to a sequence within the loop of the hairpin adaptor can be used for PCR (Figure 1C and 1D, column 5, lines 1-3, column 9, lines 8-10). Weissmen also discusses the use of abasic sites, hairpin structures, or protein binding sites on the adaptor sequences that either slow, partially obstruct, or completely block a DNA polymerase (column 3-4 and column 7-8).

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claim 2 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ahern (U.S. 5,470,724) in view of Liu et al., "Truncated Amplification: A Method for High-Fidelity Template-Driven Nucleic Acid Amplification," Biotechniques, July 2002, Vol.33, pp.129-138.

Ahern describes a method of amplifying DNA sequences of interest by cleaving DNA to produce discrete fragments, ligating the fragments to adaptor polynucleotides having a ligatable end, and first and second self complementary sequences separated by a spacer sequence, thereby forming ligated duplexes and amplifying the ligated duplexes with a polymerase (abstract, Figures 2B, 3A-4, column 2, lines 41-50). The adaptor polynucleotides are called "panhandled" and comprises of ends with complementary sequences and a spacer region inbetween that forms a loop (column 5, lines 8-14) and such an amplification is called "Boomerang DNA Amplification" or "BDA" (entire document). Primers anneal to the primer target sites on the BDA templates and are extended using a polymerizing agent (column 3, lines 8-10).

Ahern does not discuss the method wherein the panhandled adaptors comprise sequences within the spacer region that cannot be amplified by PCR.

Liu et al. describe a method of truncating DNA polymerase elongation using modified nucleotides incorporated into the template sequence.

One of ordinary skill in the art would have been motivated to modify the method of Ahern to use modified nucleotides that cannot be amplified by PCR because Liu et al. state that using modified nucleotides to truncate polymerase elongation produces truncated terminal products that are not templates for further amplification, and the products are never more than three rounds of replication from the original template, providing error-free products by dramatically limiting the propagation of DNA polymerase errors (page 136, TA versus PCR). Thus, the skilled artisan would have had a reasonable expectation of success in using modified nucleotides that inhibit DNA polymerase elongation in the method of Ahern. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed modified nucleotides that inhibit DNA polymerase elongation therein.

8. Claims 6-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Weissman et al. (U.S. 6,235,502) in view of Wagner (U.S. 6,114,115).

The teachings of Weissman are discussed above. Although Weissman also discusses using a polymerase that has 3' →5' exonuclease activity capable of removing misincorporated nucleotides, thereby correcting base mispairs (column 3, lines 44-45; column 9, lines 16-20), he does not particularly discuss converting the PCR products into hairpin structures by a method which induces denaturation followed by sudden renaturation, identifying hairpins containing mismatches or mutations, removing them,

and collecting the DNA containing no mismatches [claim 6]. Weissman also does not discuss a method wherein the concentration of primers are either equal to each other or unbalanced [claim 14]. He does not teach using the method to increase the fidelity of an assay that relies on PCR-amplified nucleic acid template, such as mutation detection, mutation analysis, polymorphism detection, polymorphism analysis, microsatellite analysis, or cloning and protein functional analysis, wherein such mutation or polymorphism detection methods are selected from the group consisting of PCR, PCR/RE/LCR, MutEx-ACB-PCR, RFLP analysis, APRIL-ATM [claims 15-17].

Wagner describes a method using an immobilized DNA mismatch-binding protein, such as MutS, to isolate or remove duplex DNA molecules containing mismatches such as error-containing molecules in PCR-amplified DNA samples (abstract). The method comprises (a) subjecting amplified DNA to conditions of denaturation followed by reannealing, such that the error-containing or the minority sequences form heteroduplexes containing a mismatch, thereby generating a mixture of perfectly matched duplexes and the heteroduplexes, (b) including the mixture with an immobilized mismatch binding protein wherein the heteroduplexes bind to the protein, and (c) removing the immobilized protein, thereby removing the majority of the sequences containing sequence errors from the amplified DNA sample (column 9, lines 12-35). In part (b) of the method, Wagner also further explains that the denatured/ reannealed DNA is in a state wherein incorrect bases are found in mismatched base pairs after annealing [i.e. conditions which create hairpin structures] (column 27, lines 10-13). In his method, the material which does not bind to the mismatch binding

proteins (MBP) is recovered, and contains only those duplex sequences without mismatches (column 6, lines 48-53). In one embodiment, Wagner also describes template strands which comprise trinucleotide repeats, wherein a secondary structure, in particular, a hairpin structure with complementary heteroduplexes, can bind to immobilized MBPs such as MutS after denaturation / renaturation (column 31-32, and column 49, lines 3-12). Denaturing, followed by reannealing for homozygous and heterozygous DNA occurs under conditions which comprise treating the amplified products to 100°C for 4 minutes, followed by 50°C for 60 minutes (column 46, lines 65-67). Conditions for DNA comprising triplet repeat sequences comprise heating the DNA to 70°C for 10 minutes, followed by cooling for 45 minutes at room temperature, and then cooled further at 4°C (column 48, lines 45-47). In several experiments, Wagner discusses PCR amplification using primers at unequal concentrations, 0.1uM primer #1, 0.075 uM primer #2 (column 41, lines 26-27), as well as at equal concentrations, 0.2 uM primer#1, 0.2 uM primer#2 (column 41, lines 65-66). Wagner describes using the method to eliminate all DNA molecules with sequence alterations introduced by PCR copy errors for accurate mutation detection, or reducing the risk of PCR amplifying a nucleotide sequence different from the starting sequence in cloning experiments (column 26, lines 40-54).

One of ordinary skill in the art would have been motivated to modify the method of Weissman to use MutS to remove mismatch duplex DNA molecules, to use primers at equal or unequal concentrations, and use the method to increase the fidelity of an assay that relies on PCR-amplified nucleic acid template, such as mutation detection via

PCR because Wagner states that PCR suffers from an inherent tendency of the polymerases to make mistakes by inserting incorrect, non-complementary bases during synthesis, and using an immobilized MBP to remove a major proportion of error-containing sequences from PCR amplified material results in relative (and possibly complete) purification of amplified DNA (column 26, lines 24-27, and 34-37). Wagner also demonstrates the benefits of using primers at equal or unequal concentrations in his examples, as well as using the method to ensure for accurate mutation detection or cloning via PCR. Therefore, the skilled artisan would have had a reasonable expectation of success in using MutS to remove hairpin DNAs containing polymerase generated mismatched nucleotides, using primers at equal or unequal concentrations, and using the method to increase the fidelity of mutation detection or cloning in the method of Weissman. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed MutS to remove mismatch duplex DNA molecules, primers at equal or unequal concentrations, and using the method to increase the fidelity of mutation detection or cloning therein.

9. Claims 6-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ahern (U.S. 5,470,724) in view of Wagner (U.S. 6,114,115).

The teachings of Ahern are discussed above. Ahern also discusses using the method for a particular application (i.e. further characterizing the PCR amplified products), such as cleaving the amplified DNA using one or more restriction nucleases and subjecting them to restriction mapping analysis on gels (i.e. RFLP) (column 12,

lines 11-14, and column 27, lines 10-65), and ligating the amplified sequence of interest into a cloning vector for sequence analysis (column 12, lines 17-20, and columns 29-31). Ahern also discusses using unequal concentrations of primers during BDA and PCR reactions, specifically 2 ug of 21-base primer and 2.7 ug of 30-base primer (column 23, lines 30-31).

Ahern does not discuss converting the PCR products into hairpin structures by a method which induces denaturation followed by sudden renaturation, identifying hairpins containing mismatches or mutations, removing them, and collecting the DNA containing no mismatches [claim 6].

The teachings of Wagner are discussed above, wherein Wagner teaches subjecting amplified PCR products to denaturation, followed by sudden renaturation (i.e. thereby creating hairpin duplex structures), and then using an enzyme (MutS) to identify and remove the sequence structures containing mismatches or mutations, thereby collecting DNA containing no mismatches.

One of ordinary skill in the art would have been motivated to modify the method of Ahern to use MutS to remove mismatch duplex DNA molecules prior to a method, such as RFLP or cloning because Wagner states that PCR suffers from an inherent tendency of the polymerases to make mistakes by inserting incorrect, non-complementary bases during synthesis, and using an immobilized MBP to remove a major proportion of error-containing sequences from PCR amplified material results in relative (and possibly complete) purification of amplified DNA (column 26, lines 24-27, and 34-37), which can ensure for accurate mutation detection or cloning via PCR.

Therefore, the skilled artisan would have had a reasonable expectation of success in using MutS to remove hairpin DNAs containing polymerase generated mismatched nucleotides prior to RFLP or cloning analysis in the method of Ahern. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed MutS to remove mismatch duplex DNA molecules therein.

10. Claim 2 is rejected under 35 U.S.C. 103(a) as being unpatentable over Abarzua (US 2003/0108902 A1) in view of Liu et al., "Truncated Amplification: A Method for High-Fidelity Template-Driven Nucleic Acid Amplification," Biotechniques, July 2002, Vol.33, pp.129-138.

Abarzua discusses a method comprising ligating hairpin oligonucleotides to a restriction enzyme fragment, or non-circular duplex DNA with strands W and C and having a single-stranded overhang at each end such that after hybridization with hairpin oligonucleotides, and subsequent ligation, the ligated product is then amplified via rolling circle amplification (RCA). Following RCA, the products can be duplexed by self-annealing, and then cut by the corresponding restriction nuclease(s) to generate the original fragment (page 2, paragraph [0024], and Figure 4). Such hairpin oligonucleotides may be designed to incorporate a binding site for universal primer sequences useful in RCA (page 8, paragraph [0070] and page 10, claims 19-20). Abarzua describes subjecting the products to 100°C (heat denaturation), followed by

quick cooling to 4°C in water/ice bath, following the reactions (page 8, paragraph [0075]).

Abarzua does not discuss the method wherein the hairpin oligonucleotide comprise sequences within the spacer region that cannot be amplified by PCR.

Liu et al. describe a method of truncating DNA polymerase elongation using modified nucleotides incorporated into the template sequence.

One of ordinary skill in the art would have been motivated to modify the method of Abarzua to use modified nucleotides that cannot be amplified by PCR because Liu et al. state that using modified nucleotides to truncate polymerase elongation produces truncated terminal products that are not templates for further amplification, and the products are never more than three rounds of replication from the original template, providing error-free products by dramatically limiting the propagation of DNA polymerase errors (page 136, TA versus PCR). Thus, the skilled artisan would have had a reasonable expectation of success in using modified nucleotides that inhibit DNA polymerase elongation in the method of Abarzua. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed modified nucleotides that inhibit DNA polymerase elongation therein.

11. Claims 6-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Abarzua (US 2003/0108902 A1) in view of Wagner (U.S. 6,114,115).

The teachings of Abarzua are discussed above. Abarzua does not discuss identifying hairpins containing mismatches or mutations, removing them, and collecting the DNA containing no mismatches [claim 6]. Abarzua also does not discuss a method wherein the concentration of primers are either equal to each other or unbalanced [claim 14]. He does not teach using the method to increase the fidelity of an assay that relies on PCR-amplified nucleic acid template, such as mutation detection, mutation analysis, polymorphism detection, polymorphism analysis, microsatellite analysis, or cloning and protein functional analysis, wherein such mutation or polymorphism detection methods are selected from the group consisting of PCR, PCR/RE/LCR, MutEx-ACB-PCR, RFLP analysis, APRIL-ATM [claims 15-17].

The teachings of Wagner are discussed above, wherein Wagner teaches subjecting amplified PCR products to denaturation, followed by sudden renaturation (i.e. thereby creating hairpin duplex structures), and then using an enzyme (MutS) to identify and remove the sequence structures containing mismatches or mutations, thereby collecting DNA containing no mismatches. Wagner discusses using primers at unequal and equal concentrations, as well as using the method to eliminate all DNA molecules with sequence alterations introduced by PCR copy errors for accurate mutation detection, or reducing the risk of PCR amplifying a nucleotide sequence different from the starting sequence in cloning experiments (also discussed above).

One of ordinary skill in the art would have been motivated to modify the method of Abarzua to use MutS to remove duplexed self-annealed products that contain mismatches or mutations, to use primers at equal or unequal concentrations, and use

the method to increase the fidelity of an assay that relies on PCR-amplified nucleic acid template, such as mutation detection via PCR because Wagner states that PCR suffers from an inherent tendency of the polymerases to make mistakes by inserting incorrect, non-complementary bases during synthesis, and using an immobilized MBP to remove a major proportion of error-containing sequences from PCR amplified material results in relative (and possibly complete) purification of amplified DNA (column 26, lines 24-27, and 34-37). Wagner also demonstrates the benefits of using primers at equal or unequal concentrations in his examples, as well as using the method to ensure for accurate mutation detection or cloning via PCR. Therefore, the skilled artisan would have had a reasonable expectation of success in using MutS to remove hairpin DNAs containing polymerase generated mismatched nucleotides, using primers at equal or unequal concentrations, and using the method to increase the fidelity of mutation detection or cloning in the method of Abarzua. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed MutS to remove mismatch duplex DNA molecules, primers at equal or unequal concentrations, and using the method to increase the fidelity of mutation detection or cloning therein.

SUMMARY

12. No claims are free of the prior art.

14. James et al., "Surprising fidelity of template-directed chemical ligation of oligonucleotides," Chemistry and Biology, Aug.1997, Vol. 4, pp. 595-605, is noted as a reference of interest.

CONCLUSIONS

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Molly E. Baughman whose telephone number is 571-272-4434. The examiner can normally be reached on Monday-Friday 8-5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Molly E Baughman
Examiner
Art Unit 1637 MEB 1/22/07

KENNETH R. HORLICK, PH.D
PRIMARY EXAMINER
1/22/07